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ORIGINAL PAPER

Growth response of three plantation species of the tropics exposed to elevated CO_2 levels

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Abstract: The response of forest trees, the largest carbon sinks on the earth, to continuing rise in atmospheric carbon levels is unknown. Reports state that increasing levels of atmospheric CO_2 will stimulate photosynthesis and productivity in most ecosystems. However, the duration and magnitude of this stimulation, particularly in the tropics, remains a question. To investigate the effects of CO_2 fertilization on plant growth, seedlings of three common plantation species, *Casuarina equisetifolia, Ailanthus excelsa* and *Tectona grandis* were grown in closed chambers enriched with CO_2. After 180 days of treatment, morphological traits of seedling height, biomass of root and shoot and root-shoot allometric co-efficient were measured. The activity of carbonic anhydrase and contents of chlorophylls, total carbohydrates and soluble proteins were determined. In *Tectona grandis*, significant effects of CO_2 supply were found on chlorophylls, root-shoot allometric ratio and seedling quality index. *Ailanthus excelsa* showed significant effect on only the shoot characteristics on exposure to elevated CO_2 but the root characteristics and concentrations of chlorophylls were not significantly different. *Casuarina equisetifolia* also showed significant effects on exposure to elevated CO_2 in terms of shoot characteristics and concentrations of chlorophylls. Total sugars, the major photosynthates, did not show any significant variation to elevated CO_2 in any of the three species. Carbonic anhydrase, the key enzyme responsible for transfer of CO_2 into the tissues significantly increased in all three species. Overall, all the variables responded to elevated CO_2, reflecting the positive effects of one parameter of climate change conditions on seedling quality. A positive response of these three plantation species to elevated CO_2 content is a good indication for their future existence in potentially changed climatic conditions.

Keywords: Plantation; Elevated CO_2; Tropical trees; India; Carbonic anhydrase

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Introduction

Human activities are causing a steady rise in carbon dioxide concentration (CO_2) in the atmosphere (IPCC 2001). CO_2 elevation can lead to changes in physiological and growth activities of plants, and consequently, changes in the biosphere (Eichelmann et al. 2004). Considerable attention has been devoted to plant physiological and growth responses to elevated CO_2 (Rey and Jarvis 1998; Roberntz and Stockfors 1998; Rogers and Humphries 2000; Jach and Ceulemans 2000; Zhang and Dang 2005; Karnosky et al. 2005; King et al. 2005; Cao et al. 2007; Kubiske et al. 2007). Growth rates usually accelerate when terrestrial plants are grown in CO_2 enriched atmospheres. The net photosynthetic rate of trees generally increases in response to CO_2 elevation if there are no other limiting environmental factors (Karnosky et al. 2005). Transient effects of elevated CO_2 on plant growth have been correlated with changes of net photosynthesis and attributed to altered levels of chlorophyll (a+b), Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) and various other photosynthetic proteins (Sage 2002). However, this growth stimulation typically subsides within a few days or weeks as plants acclimate to the elevated CO_2 treatment (Sage 1994). The acclimation of growth and photosynthesis to enhanced CO_2 is usually less pronounced in seedlings than in larger, older plants (Sage 2002; Geiger et al. 1998). Therefore, seedlings and developing tissues are important tools for studying plant responses to CO_2 enrichment. This study investigated the responses of seedling traits and primary metabolites in three tropical plantation species under the current ambient and doubled CO_2. It was hypothesized that different species respond differently to elevated CO_2 levels initially but adapt to the changed environment over a period of time.

Materials and methods

Materials

The experiment was carried out at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, India. Seedlings of each of the selected tree species namely *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* were directly sown in hycopots filled with soil : sand : farmyard manure at 2:1:1 and placed inside polytunnels made of polythene sheets of 500 microns thickness. CO_2 enrichment was done to elevate the concentration (to 640 $\mu L \cdot L^{-1}$) using CO_2 cylinders. Seedlings placed in polytunnels under ambient (365 $\mu L \cdot L^{-1}$) CO_2 served as controls. The enrichment was done on daily basis and CO_2 levels were monitored using a Portable Photosynthesis System of CID, Inc., USA. Seedlings in polytunnels without CO_2 enrichment served as control. The experiment lasted 180 days. Observations were recorded on the 90th and 180th days following treatment.

Plant growth

The experiments were carried out in five replicates of each treatment. The seedlings were selected randomly from each treatment. Each seedling was separated into shoot and root. The harvested above- and below-ground plant materials were weighed. Root traits including total root length, root fresh weight and root-collar diameter, and shoot traits including height of plant and shoot fresh weight were measured. The seedlings were then oven-dried at 70°C for 48 h. Seedling Quality Index was quantified using the method of Dickson et al. (1960) and root to shoot allometric coefficient was calculated from paired measurements of root and shoot biomass. The amount of biomass accumulated during the six-month experiment was used as a measure of growth.

Biochemical analysis

Five seedlings were sampled from each set. Sample extraction was carried out with different types of solvents for various analyses (phosphate buffer, hydrolyzed 2.5 N HCl and 80% acetone) centrifuged and then supernatant was taken for the estimation. Leaf tissue was analysed for total carbohydrates by use of the Yemm and Fokes (1954) method. A 100-mg sample was digested in 2.5 N HCl and the resulting green color was read at 630 nm in a Systronics UV-visible spectrophotometer. Total soluble proteins were measured in the leaves using the method of Lowry et al. (1951). Chlorophylls were extracted into solution with 80% acetone, and absorbance measured at 645, 652 and 663 nm to determine the total chlorophyll, chlorophylls a and b contents (Yoshida et al. 1976). Carbonic anhydrase activity was measured following Wilbur and Anderson (1948).

Data analysis

Data were analyzed using analysis of variance (ANOVA) with SPSS statistics package. Means were compared using DMRT where ANOVA showed a significant effect. Replications were also considered as variates since the seedlings did not exhibit homogeneity in growth performance due to segregation. The percentage variation of each parameter due to increased CO_2 at different growth stages was calculated over ambient level values and the maximum responsive growth stage to different levels of CO_2 was identified.

Results

Root characteristics

The results are presented here as the response of the different species in growth and chemical composition to elevated levels of CO_2, i.e. 640 $\mu L \cdot L^{-1}$ at different time intervals namely three and six months (90 and 180 days following treatment).

Tectona grandis: Root length increased throughout the growth period studied. Root length after 180 days reached a maximum of 33.36 cm. Root length of treated samples was greater than that of controls but the difference was not significant (Tables 1 and 5). The increment in root length following exposure to elevated CO_2 was 12% with 640 $\mu L \cdot L^{-1}$, over the control recorded at 180 days of growth (Fig. 1 and Table 5). ***Ailanthus excelsa***: Though root length increased throughout the growth period (Tables 2 and 4), the increment following exposure to elevated CO2 was only 0.77% over the control (Fig. 1 and Table 5). The root length after 180 days reached a maximum of 15.13 cm (Table 4). Under elevated CO2, mean root length exceeded that of controls but not significantly (Tables 2 and 5). Compared to the root length at 90 days, the root length doubled at 180 days (Table 4). ***Casuarina equisetifolia***: Elevated CO_2 did not significantly influence root length (Tables 3 and 5). Furthermore, mean root length in *Casuarina equisetifolia* following exposure to elevated CO_2 was 16% below the control (Fig. 1).

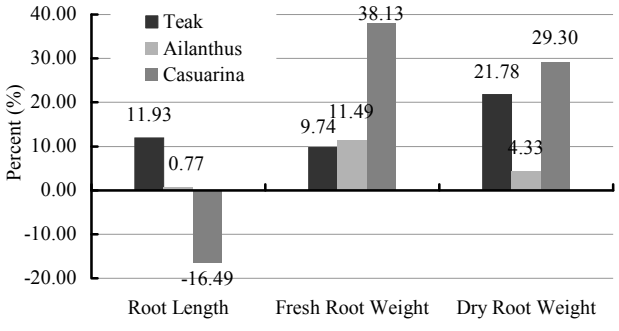

Fig. 1 Percent variation in root characteristics ratio of *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* under elevated CO_2 levels and ambient conditions

Table 1. ANOVA for various growth, biomass and chemical characteristics in *Tectona grandis* seedlings over 180 days and their response to elevated CO_2 levels (640 μL·L^{-1}) and ambient (365 μL·L^{-1}) conditions

	Replication	CO_2 levels	Time interval	CO_2×Time intervals	Error
CHLA	0.211	8.489**	28.346**	8.672**	0.370
CHLB	0.388	16.946**	25.200**	22.282**	0.206
TCHL	2.545*	10.210**	3.689**	14.603**	0.225
CA	5.914	1.022*	69.714**	1.181	0.811
RL	12.656	32.004	2374.020**	5.724	30.668
SL	4.692	10.658	6610.248**	36.992	41.781
FRW	7.115	0.772	245.210**	0.002	2.103
FSW	0.702	0.623	234.955**	0.749	1.090
DRW	1.616	0.612	52.618**	0.283	0.448
DSW	0.578	0.506	49.487**	0.450	0.169
SQI	0.097	4.131**	11.720**	6.555**	0.476
PTN	1.163*	0.105	12.593**	0.025	0.086
SUG	53.015	80.722	1700.906**	196.564*	46.673
RSRATIO	0.020	5.429**	16.928**	5.555**	0.520

** significant at p <0.01, * significant at p <0.05. CHLA: Chlorophyll a, CHLB: Chlorophyll b, TCHL: Total Chlorophyll, CA: Carbonic Anhydrase, RL: Root length, SL: Shoot length, FRW: Fresh Root weight, FSW: Fresh Shoot weight, DRW: Dry Root weight, DSW: Dry Shoot weight, SQI: Seedling Quality Index, PTN: Proteins, SUG: Sugars, RSRATIO: Root Shoot Ratio.

Table 2. ANOVA for various growth, biomass and chemical characteristics in *Ailanthus excelsa* seedlings over 180 days and their response to elevated CO_2 levels (640 μL·L^{-1}) and ambient (365 μL·L^{-1}) conditions

	Replication	CO_2 levels	Time interval	CO_2×Time intervals	Error
CHLA	13.889**	0.325	44.342**	0.684	1.184
CHLB	27.506**	2.387	68.265**	0.886	2.088
TCHL	21.477**	1.090	10.440*	0.389	1.659
CA	1.112*	5.418*	10.325**	6.601**	0.154
RL	4.624	0.041	227.813*	0.364	12.121
SL	47.742	143.112*	2453.112**	38.921	13.765
FRW	0.357	0.023	1.201*	0.015	0.113
FSW	4.316	9.647*	141.885**	1.879	2.150
DRW	0.035	0.495	0.459**	0.007	0.014
DSW	0.291	1.250*	13.284**	0.487	0.195
SQI	0.885	1.842	1.008	21.611*	2.677
PTN	1.685	6.868*	36.396**	9.086**	0.957
SUG	308.414	241.165	10130.9*	67.308	290.538
RSRATIO	2.545	0.772	2.731	27.168**	2.964

** significant at p <0.01, * significant at p <0.05. CHLA: Chlorophyll a, CHLB: Chlorophyll b, TCHL: Total Chlorophyll, CA: Carbonic Anhydrase, RL: Root length, SL: Shoot length, FRW: Fresh Root weight, FSW: Fresh Shoot weight, DRW: Dry Root weight, DSW: Dry Shoot weight, SQI: Seedling Quality Index, PTN: Proteins, SUG: Sugars, RSRATIO: Root Shoot Ratio.

Table 3. ANOVA for various growth, biomass and chemical characteristics in *Casuarina equisetifolia* seedlings over 180 days and their response to elevated CO_2 levels (640 μL·L^{-1}) and ambient (365 μL·L^{-1}) conditions

	Replication	CO_2 levels	Time interval	CO_2×Time intervals	Error
CHLA	1.06	4.223*	6.555*	2.346	0.81
CHLB	298.061*	267.034*	1527.402**	275.727*	61.817
TCHL	241.277*	202.63*	960.221**	225.792*	47.695
CA	0.154	8.765**	405.36**	0.564	0.266
RL	38.025	41.76	2086.925**	65.885	21.922
SL	52.67	276.024*	12000.101**	233.244*	37.311
FRW	0.0001225	0.06498	2.204**	0.07938	0.02885
FSW	0.861	4.168*	74.151**	3.952*	0.49
DRW	0.002103	0.01058	0.599**	0.00968	0.008404
DSW	0.31	0.865*	18.432**	0.824*	0.136
SQI	0.949	1.782	141.459**	0.812	0.652
PTN	0.495	2.513*	37.074**	0.06384	0.285
SUG	0.686	64.046	180.3*	258.696**	25.014
RSRATIO	3.312	5.335	34.087**	0.005445	1.943

Chlorophyll a, CHLB: Chlorophyll b, TCHL: Total Chlorophyll, CA: Carbonic Anhydrase, RL: Root length, SL: Shoot length, FRW: Fresh Root weight, FSW: Fresh Shoot weight, DRW: Dry Root weight, DSW: Dry Shoot weight, SQI: Seedling Quality Index, PTN: Proteins, SUG: Sugars, RSRATIO: Root Shoot Ratio.

Root fresh and dry weights of *Tectona grandis*: Both root fresh and dry weights followed the same trend as root length. The percentage increase over control was about 9.74% in root fresh weight and 21.78 % in root dry weight (Fig. 1; Table 5). ***Ailanthus excelsa***: Elevated CO_2 did not significantly influence the fresh and dry weights, but percentage increase over control was about 11.49% in root fresh weight and 4.33 % in root dry weight (Fig. 1; Table 5). ***Casuarina equisetifolia***: The trend observed in *Tectona grandis* and *Ailanthus excelsa* was observed in this species. The percent increment over the control was recorded as 38.13 and 29.30 for fresh and dry weights, respectively (Fig. 1; Table 5).

Shoot characteristics

Shoot length of *Tectona grandis*: Shoot length was shorter in elevated conditions of CO_2 when compared with controls, the difference being 5% less than controls after 180 days of growth (Fig. 2; Table 5). ***Ailanthus excelsa***: CO_2 significantly increased the shoot length of *Ailanthus excelsa* (Table 2). The combined effects of growth period and CO_2 did not influence the plant height significantly (Table 2). The increment was 22.94% greater than for controls. This was the highest recorded among the three species studied. ***Casuarina equisetifolia***: Similar to *Ailanthus excelsa*, elevated CO_2 significantly influenced the shoot length in *Casuarina equisetifolias* (Tables 3 and 5). The

increment in shoot length in *Casuarina equisetifolia* following exposure to elevated CO_2 was 21.74 percent.

Table 4. Various growth, biomass and chemical characteristics in *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* seedlings over 90 and 180 days in closed chambers.

Parameters	*Tectona grandis*		*Ailanthus excelsa*		*Casuarina equisetifolia*	
	3 months	6 months	3 months	6 months	3 months	6 months
CHLA	1.48±0.08	3.86±1.59	1.66±0.20	4.64±1.88	1.51±0.33	2.65±1.45
CHLB	1.72±0.43	3.96±2.14	1.54±0.61	5.23±2.56	14.02±0.13	18.35±0.87
TCHL	3.20±0.40	4.06±1.80	3.20±0.43	4.64±2.27	12.40±0.40	16.24±2.38
CA	1.18±0.50	4.92±1.42	1.49±0.43	2.93±1.24	11.36±0.97	12.35±0.74
RL	11.57±2.41	33.36±7.13	8.38±2.52	15.13±3.80	5.87±0.89	26.30±7.21
SL	10.21±1.51	46.57±8.55	14.92±3.10	37.07±6.24	13.40±2.32	62.39±10.92
FRW	0.73±0.27	7.73±2.08	0.38±0.11	0.87±0.47	0.02±0.01	0.69±0.25
FSW	1.07±0.19	7.93±1.42	1.48±0.46	6.81±2.26	0.05±0.02	3.90±1.35
DRW	0.16±0.08	3.40±1.01	0.06±0.03	0.36±0.16	0.01±0.00	0.35±0.13
DSW	0.39±0.08	3.54±0.67	0.38±0.11	2.01±0.73	0.02±0.01	1.94±0.67
SQI	1.57±0.28	3.10±1.38	6.12±2.41	6.57±1.16	0.32±0.19	5.64±1.20
PTN	1.50±0.26	3.08±0.47	2.93±0.23	5.63±1.87	1.94±0.67	4.66±0.61
SUG	33.39±4.12	51.83±9.87	34.58±3.88	79.59±23.19	26.08±6.24	32.08±6.22
RSRATIO	1.10±0.28	2.94±1.42	5.94±2.42	6.68±1.57	3.00±1.66	5.61±1.20

CHLA: Chlorophyll a, CHLB: Chlorophyll b, TCHL: Total Chlorophyll, CA: Carbonic Anhydrase, RL: Root length, SL: Shoot length, FRW: Fresh Root weight, FSW: Fresh Shoot weight, DRW: Dry Root weight, DSW: Dry Shoot weight, SQI: Seedling Quality Index, PTN: Proteins, SUG: Sugars, RSRATIO: Root Shoot Ratio.

Table 5. Various growth, biomass and chemical characteristics in *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* seedlings over 180 days and their response to elevated CO_2 levels (640 μL·L^{-1}) and ambient (365 μL·L^{-1}) conditions

Parameters	*Tectona grandis*		*Ailanthus excelsa*		*Casuarina equisetifolia*	
	Ambient	Elevated CO_2	Ambient	Elevated CO_2	Ambient	Elevated CO_2
CHLA	2.02±0.79	3.32±2.03	3.15±1.95	3.15±2.17	1.62±0.61	2.54±1.45
CHLB	1.92±0.55	3.76±2.31	3.73±2.19	3.04±3.07	5.96±7.59	13.26±16.70
TCHL	2.91±0.86	4.34±1.40	4.15±1.32	3.69±2.15	6.13±6.10	12.49±14.17
CA	2.82±2.29	3.27±2.15	1.69±0.47	2.73±1.43	6.19±4.58	7.52±4.96
RL	21.20±12.26	23.73±12.90	11.71±5.14	11.80±4.44	17.53±13.47	14.64±9.92
SL	27.66±18.50	29.12±21.60	23.32±10.54	28.67±13.93	34.18±23.22	41.61±29.80
FRW	4.04±3.95	4.43±3.99	0.59±0.52	0.66±0.31	0.30±0.32	0.41±0.44
FSW	4.32±3.55	4.68±3.94	3.45±3.15	4.84±3.18	1.52±1.76	2.43±2.54
DRW	1.61±1.68	1.96±2.00	0.21±0.23	0.22±0.16	0.16±0.18	0.20±0.22
DSW	1.81±1.54	2.12±1.87	0.95±0.89	1.45±1.05	0.77±0.90	1.19±1.26
SQI	1.88±0.30	2.79±1.66	6.04±2.06	6.65±1.68	2.68±2.67	3.28±3.14
PTN	2.22±0.87	2.36±0.96	3.70±1.09	4.87±2.38	2.94±1.60	3.65±1.44
SUG	40.60±8.91	44.62±14.66	53.61±27.35	60.56±30.06	27.29±6.23	30.87±7.19
RSRATIO	1.50±0.48	2.54±1.77	6.12±2.39	6.51±1.68	3.79±1.57	4.82±2.22

CHLA: Chlorophyll a, CHLB: Chlorophyll b, TCHL: Total Chlorophyll, CA: Carbonic Anhydrase, RL: Root length, SL: Shoot length, FRW: Fresh Root weight, FSW: Fresh Shoot weight, DRW: Dry Root weight, DSW: Dry Shoot weight, SQI: Seedling Quality Index, PTN: Proteins, SUG: Sugars, RSRATIO: Root Shoot Ratio.

Shoot fresh and dry weights of *Tectona grandis*: *Tectona grandis* showed increase in shoot fresh and dry weights over growth periods (Tables 1 and 4). CO_2 did not influence the fresh and dry weights significantly (Tables 1 and 5) but increments over the controls were 8.17% and 17.62%, respectively). ***Ailanthus excelsa***: Similar to shoot length, CO_2 had significant influence on shoot fresh and dry weights (Table 2). The combined effect of CO_2 and growth period had no significant influence on the fresh and dry weights. The percentage increase over control was about 40.27% in shoot fresh weight and 52.91% in shoot dry weight under 640 μL·L^{-1}. (Fig. 2; Table 5). ***Casuarina equisetifolia***: The trend observed for *Ailanthus excelsa* was also observed for *Casuarina equisetifolias* (Table 3). The percentage increase over control in shoot fresh weight was 60.03% and 54.10% in shoot

dry weight under 640 μL·L^{-1} (Fig. 2; Table 5)..

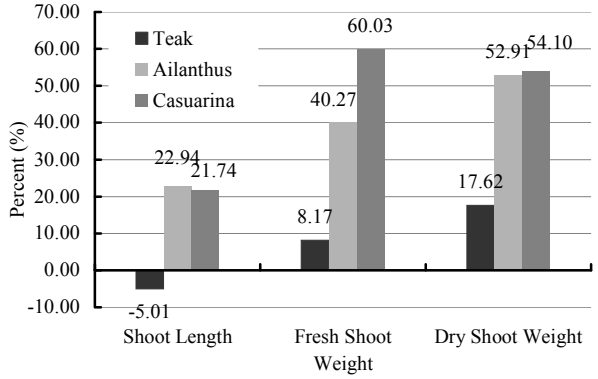

Fig. 2 Percent variation in shoot characteristics of *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* under elevated CO_2 levels and ambient conditions

Total biomass

Elevated CO_2 levels enhanced the total biomass at higher levels of CO_2. The increment in total biomass was 19.58% in *Tectona grandis*, 44.51% in *Ailanthus excelsa* and 49.89% in *Casuarina equisetifolia* over their respective controls. *Casuarina equisetifolia* showed the greatest biomass increment in response to elevated CO_2 levels (Fig. 3).

Root / shoot (R/S) ratio

Tectona grandis seedlings showed significant variation in root:shoot ratio (R/S) with respect to CO_2 level, growth period and interaction between the two main factors (Table 1), while *Casuarina equisetifolia* showed significant increase over periods of time. *Ailanthus excelsa* showed significant variation with respect to combined effects of CO_2 elevations and growth periods but not individually. The increments observed in root:shoot ratio in the species were 69.3, 6.4 and 27.2 (Fig. 3) for *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* respectively.

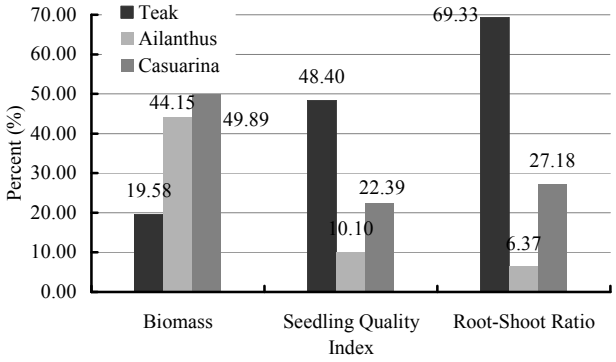

Fig. 3 Percent variation in Total biomass, seedling quality index and root/shoot ratio of *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* under elevated CO_2 levels and ambient conditions

Seedling quality index (SQI)

A trend similar to root-shoot ratio was observed in all three species. The SQI increment was highest in *Tectona grandis* (48.4 %; p <5%; Table 1) followed by *Casuarina equisetifolia* (22.39%; p >5%; Table 2) and *Ailanthus excelsa* (10.10 %; p >5%; Table 3; Fig. 3). The increase in *Tectona grandis* could be attributed to the increase in the leaf size as a result of elevated CO_2 levels. This result is in agreement with the R/S ratio.

Biochemicals

Chlorophylls of *Tectona grandis*: Chlorophylls a, b and total showed significant increase with elevated CO_2 levels and with the combined effects of growth period and elevated CO_2 levels (Tables 1 and 5). The increment in chlorophylls was 64.5%, 95.84% and 49.06%, respectively for chlorophylls a, b and total recorded at 180 days of growth (Fig. 4; Table 5). ***Ailanthus excelsa***: CO_2 had a negative influence on the chlorophylls in *Ailanthus excelsa* (Fig. 4; Table 5). The decline in chlorophylls was highest in chlorophyll b (18.52 per cent), followed by total chlorophyll (11.24 per cent). Chlorophyll a levels were similar in treated and control seedlings but slightly lower for controls (Fig. 4; Table 5). The combined effects of growth period and CO_2 did not in any way influence the chlorophylls (Table 2). ***Casuarina equisetifolia***: Elevated CO_2 and time period significantly influenced the chlorophylls in *Casuarina equisetifolia* (Table 3). Increments of chlorophylls a, b, and total were 56.66, 122.7 and 103.9, respectively (Fig. 4, Table 5). Replications showed significant increase in the chlorophyll b and total levels suggesting a need to screen genotypes to understand their varied responses. Combined effects of CO_2 and growth period showed significant responses for chlorophyll b and total chlorophyll (Table 3).

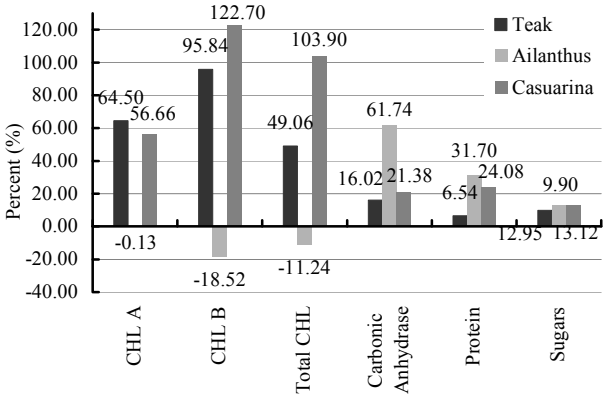

Fig. 4 Percent variation in biochemicals namely chlorophylls, CA activity, total sugars and proteins of *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* under elevated CO2 levels and ambient conditions

Carbonic anhydrase: CA increased significantly in *Casuarina equisetifolia*, *Tectona grandis* and *Ailanthus excelsa* in response to elevated CO_2 (Tables 1 to 3). The increments were

16.02%, 61.74% and 21.38%, respectively for *Tectona grandis*, *Ailanthus excelsa* and *Casuarina equisetifolia* (Fig. 4; Table 5).

Sugars and proteins: Sugars did not show significant responses to increased levels of CO_2 though treatment means were higher for all three species, the increments being 10.0, 12.95 and 13.12, respectively. However, protein levels varied significantly in *Casuarina equisetifolia* and *Ailanthus excelsa*, with recorded increments of 31.7 and 24.8 (Fig. 4; Table 5).

Discussion

Elevated levels of atmospheric CO_2 can increase production in greenhouses (Mortensen, 1987). Effects on gas exchange, respiration, growth, and development have been documented for a variety of plant species (Curtis and Wang 1998). Use of elevated CO_2 in the greenhouse, therefore, can be used to modify the physiology, size, or morphology of plants in order to meet specific objectives relevant to the desired end use (e.g. afforestation, agroforestry, reclamation). We subjected three tropical species to increased levels of CO_2 at the nursery stage. The most responsive was *Casuarina equisetifolia*, a nitrogen fixing species, preferred by tree farmers due to its multiple uses and short rotation. This was followed by *Ailanthus excelsa*, an indigenous fast growing tree species in use by match industries. *Tectona grandis*, a long rotation species, showed less pronounced responses than the fast-growing species. The performance of *Tectona grandis* was poor at the end of three months, indicating adverse effect of CO_2 enrichment on morphological traits of this species (Varadarajan et al. 2010) however at the end of six months, *Tectona grandis* showed higher root:shoot ratio and seedling quality index indicating better adaptability to elevated CO_2 levels. Since stump planting is the preferred propagule for planting *Tectona grandis*, higher SQI indicates that the species would be able to perform better under varied climatic conditions.

A second significant feature of the data sets is varying root:shoot ratios of the three species. Allometrics is a useful tool to evaluate biomass allocation among different plant organs. It is based on the logarithmic relationships between biomass partitioned to the two plant organs (root to shoot) (Nicklas 2005). In nature, plants are believed to develop a root to shoot ratio that is partly genetically inherited and partly determined by the environment. Plants sense the environment and respond to fluctuations in the resources availability by applying morphological and physiological controls that alter, among other processes, the carbon allocation pattern.

The root:shoot ratio is one measure that helps assess the overall health of plants. An increase in root:shoot ratio can indicate a healthier plant provided the increase is from greater root size and not from lower shoot weight. In the present study, elevated CO_2 levels maintained better root:shoot ratios than did controls. Surprisingly, *Tectona grandis* had the highest increment for root:shoot ratio with higher root biomass but low shoot biomass increment. The high root:shoot ratio and SQI suggests that *Tectona grandis*, although it did not show significant morphological responses during the study period, could adapt better to elevated CO_2 levels over longer periods of exposure. However, in *Casuarina equisetifolia* and *Ailanthus excelsa*, increases in root:shoot ratio were more attributable to increases in shoot characteristics than the root though both showed increments in their overall growth performance.

Morphological and growth responses to elevated CO_2 include increased biomass yield, height, total leaf area, total leaf weight and size, leaf weight per unit area, and dry matter allocation to roots (Radoglou and Jarvis, 1990a, b; Ceulemans and Mousseau, 1994; Curtis and Wang, 1998). In our study, there was an increase in all shoot characteristics except in *Tectona grandis* where shoot length did not show positive response. Field observations revealed larger leaf area for the species under elevated CO_2 suggesting resource allocation for expansion of leaves rather than to height. Root characteristics also showed positive response, except in root length in *Casuarina equisetifolia*, but field observations showed increased secondary and tertiary roots, this observation is in agreement with the root biomass increment. In all the three species, the biomass increased over the ambient grown species.

Aspen and poplars grown in elevated CO_2 accumulated 55% more dry mass than trees grown in ambient CO_2 conditions. This increase resulted from dry mass increase of all the tree components, i.e. stem, branches, buds, needles and roots. In work with woody species at ambient and elevated CO_2, Tischler et al. (2004) observed significant effects of elevated CO_2 on total biomass for mesquite (*Prosopis glandulosa*) at day 3, and for parkinsonia (*Parkinsonia aculeata* L.), honey locust (*Gleditsia triacanthos* L.), and huisache (*Acacia farnesiana* (L.) Willd.) at eighth day. Similar increases in dry mass in response to CO_2 enrichment have been observed for a range of tree species grown under field conditions (Norby et al. 1999). Elevated CO_2 has been found to cause greater allocation to root biomass (Dickson et al. 1998) and in our study, this was observed for all three species. An increase of 15% in stem height and 30%–45% in biomass under elevated CO_2 has been reported in tree species (Ceulemans et al. 1996; Curtis and Wang 1998; Wang et al. 2000; Zak et al. 2000). An increase in height of 8% and biomass (15%–30%) was observed in hybrid poplar under elevated CO_2 (Tupker et al. 2003).

Accumulation of foliar carbohydrates is one of the most pronounced and universal changes observed in the leaves of C3 plants grown at elevated CO_2 concentration. In our study, the levels of total carbohydrates / sugars did not show significant variation in response to elevated CO_2 levels. Rogers and Ainsworth (2006) report that trees have large sinks for photosynthates and may be expected to avoid foliar carbohydrate accumulation in the presence of elevated CO_2. Developing loblolly pines experiencing a step change in CO_2 at the Duke Forest FACE experiment (Hendrey et al. 1999) did not show an accumulation of carbohydrates when measured at multiple stages during the first season of CO_2 exposure (Myers et al. 1999). Rogers and Ellsworth (2002) did report foliar carbohydrate accumulation. Herrick and Thomas (2001) did not report carbohydrate accumulation in sun or shade leaves of *Liquidambar styraciflua* (sweetgum) growing at elevated CO_2 in the understory at the Duke

Forest FACE site (Herrick and Thomas 2001). However, Tissue et al. (2002) did report carbohydrate accumulation at elevated CO_2 in the same species at the Oak Ridge National Laboratory FACE site (Norby et al. 2001). Singaas et al. (2000) reported carbohydrate accumulation in *Acer rubrum*, *Ceris canadensis* and *L. styraciflua* at the Duke site. So it could be inferred that our understanding of the mechanisms underlying the response of foliar carbohydrates to elevated CO_2 in these tree species needs to be increased.

Further, if high-CO_2 grown plants invest relatively more in cell walls and/or secondary compounds, this may increase their leaf longevity, but slow their growth, whereas an additional investment in proteins (for example in photosynthetic machinery) may accelerate growth (Poorter and Bergkotte 1992). Here in our experiments, we observed an increase in the levels of proteins in *Casuarina equisetifolia* a major nitrogen source of the plant suggesting that the total non structural carbohydrate (TNC) levels could have increased, with an addition to the photosynthetic machinery as is evidenced by higher levels of chlorophylls.

Conclusion

Elevated CO_2 is a tool that can be used to modify growth and resource allocation in tropical tree species during nursery production prior to large-scale use. Overall, the three species responded positively to elevated CO_2 with increased growth and allocation to roots. Modification of morphology through control of atmospheric CO_2 can be combined with selection of clones to produce planting stock appropriate to the end purpose. For agroforestry/afforestation, both early establishment and maximum growth are of interest; in reclamation of ecologically degraded sites, large root systems that help ensure survival and rapid uptake of water and minerals are important. The latter would be useful even in bioremediation by absorption of contaminants.

References

Cao B, Dang QL, Zhang S. 2007. Relationship between photosynthesis and leaf nitrogen concentration under ambient and elevated (CO_2) in white birch (*Betula papyrifera*) seedlings. *Tree Physiology*, **27**(6): 891–899.

Ceulemans R, Jiang XN, Shao BY. 1995. Growth and physiology of one-year old poplar (Populus) under elevated atmospheric CO_2 levels. *Annals of Botany*, **75**(6): 609–617.

Ceulemans R, Shao BY, Jiang XN, Kalina J. 1996. First- and second-year aboveground growth and productivity of two Populus hybrids grown at ambient and elevated CO_2. *Tree Physiology*, **16**(1–2): 61–68.

Ceulemans W, Mousseau M. 1994. Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist*, **127**(3): 425–446.

Curtis PS, Wang XZ. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia*, **113**(3): 299–313.

Dickson RE, Coleman MD, Riemenschneider DE, Isebrands JG, Hogan GD, Karnosky DF. 1998. Growth of five hybrid poplar genotypes exposed to interacting elevated CO_2 and O_3. *Canadian Journal of Forest Research*, **28**: 1706–1716

Dickson A, Leaf AL, Hosner JF. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. *The Forestry Chronicle*, **36**(1): 10–13.

Eichelmann H, Oja V, Rasulov B, Padu E, Bichele I, Pettai H, Möls T, Kasparova I, Vapaavuori E, Laisk A. 2004. Photosynthetic parameters of birch (*Betula pendula* Roth) leaves growing in normal and in CO_2- and O_3- enriched atmospheres. *Plant Cell and Environment*, **27**(4): 479–495.

Ganade G, Westoby M. 1999. Seed mass and the evolution of early seedling etiolation. *American Naturalist*, **154**(4): 469–480.

Geiger M, Walch-Liu P, Engels C, Harnecker J, Schulze ED, Ludewig F, Sonnewald U, Scheible WR, Stitt M. 1998. Enhanced carbon dioxide leads to a modified diurnal rhythm of nitrate reductase activity in older plants, and a large stimulation of nitrate reductase activity and higher levels of amino acids in young tobacco plants. *Plant Cell and Environment*, **21**(3): 253–268.

Hendrey GR, Ellsworth DS, Lewin KF, Nagy J. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology*, **5**(3): 293–309.

Herrick JD, Thomas RB. 2001. No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO_2 enrichment at the Duke forest FACE experiment. *Plant Cell and Environment*, **24**(1): 53–64.

IPCC. 2001. Summary for policymakers. Report of Working Group I. Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland Available at: http://www.grida.no/climate/ipcc_tar/vol4/english/005.htm.

Jach ME, Ceulemans R. 2000. Effects of season, needle age and elevated CO_2 on photosynthesis in Scots pine (*Pinus sylvestris*). *Tree Physiology*, **20**: 145–157.

Karnosky DF, Pregitzer KS, Zak DR, Bubiske ME, Hendrey GR, Weinstein D, Nosal M, Percy KE. 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant Cell and Environment*, **28**(8), 965–981.

King JS, Kubiske ME, Pregitzer KS, Hendrey GR, McDonald EP, Giardina CP, Quinn VS, Karnosky DF. 2005. Tropospheric O_3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO_2. *New Phytologist*, **168**(3): 623–636.

Kubiske ME, Quinn VS, Marquard PE, Karnosky DF. 2007. Effects of elevated atmospheric CO_2 and/or O_3 on intra- and inter-specific competitive ability of aspen. *Plant biology*, **9**(2): 342–355.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, **193**(1): 265–275.

Maranon T, Grubb PG. 1993. Physiological basis and ecological significance of the seed size and relative growth rate relationship in Mediterranean annuals. *Functional Ecology*, **7**: 591–599.

Mortensen LM. 1987. Review: CO_2 enrichment in greenhouses, crop responses. *Scientia Horticulturae*, **33**: 1–25.

Myers DA, Thomas RB, DeLucia EH. 1999. Photosynthetic capacity of loblolly pine (*Pinus taeda* L.) trees during the first year of carbon dioxide enrichment in a forest ecosystem. *Plant Cell and Environment*, **22**(5): 473–482

Niklas KJ. 2005. Modelling below- and above-ground biomass for non-woody and woody plants. *Annals of Botany*, **95**(2): 315–321.

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R. 1999. Tree responses to rising CO_2 in field experiments: implications for

the future forest. *Plant Cell and Environment*, **22**(6): 683−714.

Norby RJ, Todd DE, Fults J, Johnson DW. 2001. Allometric determination of tree growth in a CO_2-enriched sweetgum stand. *New Phytologist*, **150**: 447–487

Poorter H, Bergkotte M. 1992. Chemical composition of 24 wild species differing in relative growth rate. *Plant, Cell and Environment*, **15**: 221−229.

Radoglou KM, Jarvis PG. 1990a. Effects of CO_2 enrichment on four poplar clones. I. Growth and leaf anatomy. *Annals of Botany*, 65(6): 617–626.

Radoglou KM, Jarvis PG. 1990b. Effects of CO_2 enrichment on four poplar clones. I. Leaf surface properties. *Annals of Botany*, 65(6): 627–632.

Rey A, Jarvis PG. 1998. Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. *Tree Physiology*, **18**: 441–450.

Roberntz P, Stockfors J. 1998. Effects of elevated CO_2 and nitrogen on net photosynthesis, stomatal conductance and needle respiration of field-grown Norway spruce trees. *Tree Physiology*, **18**: 233–241.

Rogers A, Ellsworth DS. 2002. Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO_2 (FACE). *Plant Cell and Environment*, **25**(7): 851–858.

Rogers A, Ainsworth EA. 2006. The Response of Foliar Carbohydrates to Elevated (CO_2). In: Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H (Eds.), *Managed Ecosystems and CO_2*. Berlin Heidelberg: Springer-Verlag.

Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, **83**(1–2): 155–189.

Rogers A, Humphries SW. 2000. A mechanistic evaluation of photosynthetic acclimation at elevated CO_2. *Global Change Biology*, **6**(8): 1005–1011.

Sage RF. 1994. Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. *Photosynthesis Research*, **39**: 351–368.

Sage RF. 2002. How terrestrial organisms sense, signal and respond to carbon dioxide. integrative and comparative biology, **42**(3): 469–480.

Singsaas EL, Ort DR, DeLucia EH. 2000. Diurnal regulation of photosynthesis in understory saplings. *New Phytologist*, **145**(1): 39–49

Smith CC, Fretwell SD. 1974. The optimal balance between size and number of offspring. *American Naturalist*, **108**: 499−506.

Stebbins GL. 1976a. Seed and seedling ecology in annual legumes: I. A comparison of seed size and seedling development in some annual species. *Oecologia Plantarum*, **11**: 321−331.

Stebbins GL. 1976b. Seed and seedling ecology in annual legumes. II. Stem growth, seed production and mechanisms for transport. *Oecologia Plantarum*, **11**: 333−344.

Tischler CR, Derner JD, Polley HW, Johnson HB. 2004. Responses of seedlings of five woody species to carbon dioxide enrichment. In: Hild AL, Shaw NL, Meyer SE, Booth DT, McArthur ED (eds.), *Seed and soil dynamics in shrubland ecosystems: proceedings Vol. 31*. 2002 August 12−16. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, pp. 161−163.

Tischler CR, Polley HW, Johnson HB, Pennington RE. 2000. Seedling response to elevated CO_2 in five epigeal species. *International Journal of Plant Science*, **161**: 779−783.

Tissue DT, Lewis JD, Wullschleger SD, Amthor JS, Griffin KL, Anderson OR. 2002. Leaf respiration at different canopy positions in sweetgum (*Liquidambar styraciflua*) grown in ambient and elevated concentrations of carbon dioxide in the field. *Tree Physiology*, **22**: 1157−1166

Tupker KA, Thomas BR, Macdonald SE. 2003. Propagation of trembling aspen and hybrid poplar for agroforestry: potential benefits of elevated CO_2 in the greenhouse. *Agroforestry Systems*, **59**: 61−71.

Varadharajan S, Buvaneswaran C, Warrier RR, Jayaraj RSC. 2010. Response of Important Tropical tree species to Elevated Carbon di oxide. *Indian Forester*, **136**(11): 1439−1444.

Wang S, Curtis PS, Pregitzer KS, Zak DR. 2000. Genotypic variation in physiological and growth responses of *Populus tremuloides* to elevated atmospheric CO_2 concentration. *Tree Physiology*, **20**: 1019–1028

Wilbur KM, Anderson NG. 1948. Electronic and colorimetric determination of carbonic anhydrase. *Journal of Biological Chemistry*, **176**: 147−154

Yemm EW, Fokes BF. 1954. The estimation of carbohydrates in plant extract by anthrone. *Biochemical Journal*, **57**: 509−514.

Yoshida S, Formo DA, Cock JH, Gomez KA. 1976. *Laboratory manual for physiological studies of rice (3rd Edn.)*. Los Banos, Phillipines: IRRI, p. 83.

Zak DR, Pregitzer KS, Curtis PS, Vogel CS, Holmes WE, Lussenhop J. 2000. Atmospheric CO_2, soil-N availability, and allocation of biomass and nitrogen by *Populus tremuloides*. *Ecological Applications*, **10**(1): 34–46

Zhang SR, Dang QL. 2005. Effects of soil temperature and elevated CO_2 concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. *Tree Physiology*, **25**: 609–617.